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Specificity and recognition in mycorrhizal associations

J.A. DUDDRIDGE

Department of Forestry, Oxford University,
South Parks Road, Oxford OX1 3RB, Great Britain

INTRODUCTION

The ubiquitous nature of mycorrhizas is the result of the low level of specificity that exists between most mycorrhizal fungi and their hosts. The majority of plants are resistant to plant pathogens but susceptible to mycorrhizal fungi due to opposing selection pressures. It is the selection against resistance and for susceptibility which reduces a major source of specificity. I use the term specificity here to mean selectivity, as opposed to some workers who use the term synomously with effectiveness. Although most mycorrhizal fungi have a low degree of host specificity, different symbionts may vary in their ability to increase the effectiveness of nutrient absorption by the host. Specificity when it does occur is at the genus level and above. There is no situation where a strain or even a species of fungus is found to be specific for a particular host.

SPECIFICITY IN ECTOMYCORRHIZAS

Ectomycorrhizas have always been associated with a large number of genera in the Pinaceae, Salicaceae, Betulaceae and Fagaceae in the Northern Hemisphere and *Eucalyptus* spp. and some members of the Caesalpinoideae and Dipterocarpaceae in the tropics and sub-tropics. Although mycorrhizas are widespread among the Spermatophyta only 3% were estimated to be ectomycorrhizal by MEYER (1973), the rest being endomycorrhizal. Some ectomycorrhizal hosts appear to be more restrictive towards their fungal symbionts e.g. *Alnus,* while others are more flexible. For example a growing number of plants including trees (VOZZO and HACSKAYLO, 1974), shrubs (WARCUP, 1980), perennial and annual herbs (WARCUP and McGEE, 1983) and ferns (IQBAL *et al.*, 1981) have been found to form both ecto- and endo- mycorrhizas.

Ectomycorrhizal fungi are represented in all the major groups in the Eumycota, including 25 families in the Basidiomycotina, 7 in the Ascomycotina and 1 in the Zygomycotina (*Endogone flammicorona* TRAPPE and GERD.). Recently even a *Glomus* sp, (normally a VAM genus), identified as *G. tubiforme,* has been found to form ectomycorrhizas on *Eucalyptus, Pinus* and *Quercus* spp. (WARCUP, 1985a). The majority of ectomycorrhizal fungi have a very wide host range and one host may form ectomycorrhizas with a number of different fungal symbionts simultaneously (TRAPPE 1962). TRAPPE (1977) estimated that there are two thousand species of fungus that are potential ectomycorrhizal symbionts of *Pseudotsuga menziesii*. More specific ectomycorrhizal associations do occur, but specificity is always at the genus rather than the species level.

MOLINA and TRAPPE (1982b) recognised three groups of ectomycorrhizal fungi, those with broad host ranges, those with intermediate host ranges which are 'sporocarp-specific' i.e. they have specific or limited sporocarp-host associations and those which have much narrower host ranges. Broad host range fungi include species such as *Thelephora terrestris*, *Paxillus involutus*, *Amanita muscaria*, *Pisolithus tinctorius* and *Cenococcum graniforme*. Indeed *P. tinctorius* has been quoted as having "the capacity to form ectomycorrhizas on roots of all the world's tree species normally ectomycorrhizal" (MARX, 1977). *C. graniforme* has been observed to form both ectomycorrhizas with hosts that are normally endomycorrhizal and more unusually endomycorrhizas with *Stellaria holostea* (Caryophyllaceae) (see TRAPPE, 1964 for refs). In a quite extraordinary experiment, FILER and TOOLE (1966) managed to get normally ectomycorrhizal fungi eg. *C. graniforme*, *Amanita muscaria* and *Rhizopogon roseolus* to form apparently symbiotic endomycorrhizas with *Liquidambar styraciflua* in aseptic culture. Some fungi are considered to be conifer specific eg *Suillus*, *Rhizopogon* and *Truncocolumella* or even genus-specific eg. some *Rhizopogon* spp. on *Pinus* spp. (MOLINA and TRAPPE, 1982b, MALAJCZUK *et al*., 1982), *Alpova diplophloeus* on *Alnus* spp., (MOLINA, 1981) *Hydnagium carneum* on *Eucalyptus* spp. (MALAJCZUK *et al*., 1982; 1984) and *Suillus grevillei* (MELIN, 1922) and *Fuscoboletinus aeruginascens* (MOLINA and TRAPPE, 1982b) on *Larix* spp.

Suillus grevillei, , however, has been induced to form ectomycorrhizas in aseptic culture with *P. menziesii* and *Pinus sylvestris* (MOLINA and TRAPPE, 1982b; DUDDRIDGE, 1985, DUDDRIDGE in prep.) although field evidence of these associations is rare. In an ultrastructural study on associations formed between *S. grevillei* and a number of ectomycorrhizal hosts, the author (in prep.) has shown that apart from *Larix* spp. mycorrhizas were only formed with *P. menziesii* and *P. sylvestris* and in these the symbionts may not be fully compatible. It is the study of imcompatible interactions between mycorrhizal symbionts in those associations where the specificity is higher, that will give us some insight into compatibiity and recognition in ectomycorrhizas. In *P. sylvestris* a Hartig net occurs irregularly and a loose sheath is all that is formed in many cases. In ectomycorrhizas synthesised between *P. menziesii* and *S. grevillei* host wall appositions (Plate 1a) and thickening of the cell wall (Plate 1b) often develop where it is in contact with the fungus. Phenolic substances are formed at the host-fungus interface and in the fungus itself (Plate 1b). Lignification of host cell walls, loose, unstructured sheaths and little or no Hartig net development have been demonstrated in other incompatible interactions between a number of ectomycorrhizal symbionts (MOLINA and TRAPPE, 1982b; MALAJCZUK *et al*., 1984). In a few associations (eg. *Paxillus involutus*/ *Alnus* spp.; *Amanita muscaria*/*Eucalyptus* spp; *P. menziesii*/*Larix* spp.) the large increase in phenolic substances does not appear to prevent a well developed sheath and Hartig net (MOLINA and TRAPPE, 1982b; MALAJCZUK *et al*., 1984; DUDDRIDGE in prep.). It is not known whether the interface is functional in those associations where the symbionts demonstrate a tolerance to the host's response. More work needs to be done on the role of phenolic compounds in ectomycorrhizal compatibility and specificity.

Ecological specificity or the ability of many hosts to form ectomycorrhizas with a wider spectrum of symbionts in culture than in the field is at least partly (if not totally) to do with the high levels of carbohydrate used in the synthesis medium. Carbohydrate can drastically effect the nature of the association and cause changes to occur in the host-fungus interface (DUDDRIDGE and READ, 1984c; DUDDRIDGE, 1985; DUDDRIDGE, in prep.). It is therefore of paramount importance, when studying specificity and compatibility in ectomycorrhizas in aseptic culture, to omit any form of external carbohydrate from the synthesis medium.

SPECIFICITY IN MONOTROPOID MYCORRHIZAS

Monotropoid mycorrhizas are formed by achlorophyllous members of the Ericaceae, in the sub-family Monotropoideae (DUDDRIDGE and READ, 1982b; ROBERTSON and ROBERTSON, 1982). Hyphal connections have been observed between the fungal associate of these plants and ectomycorrhizal associates of nearby autotrophic hosts such as *Pinus* (BJORKMAN, 1960; DUDDRIDGE and READ, 1982b) (Plate 1c), *Salix* (DUDDRIDGE and READ, 1982b) *Abies* and *Populus* (VREELAND *et al*., 1981). Isolates from monotropoid roots include a *Boletus* sp. (BJORKMAN, 1960) and one isolate which has been shown to form ectomycorrhizas on *Picea abies* in culture (LINNEMAN, 1969). The ability of a certain group of fungi to form different mycorrhizas on hosts again underlines the lack of specificity and indicates the importance of the host in determining the structure of the mycorrhiza.

SPECIFICITY IN ARBUTOID MYCORRHIZAS

Arbutoid mycorrhizas, which possess a sheath, Hartig net and intracellular infection, are characteristic of species in the Pyrolaceae eg. *Pyrola* and some species among the Ericaceae (eg. *Arbutus* and *Arctostaphylos*). The fungal symbionts appear to be the same as those forming ectomycorrhizas on conifers. Evidence for this has come from both aseptic syntheses (MOLINA and TRAPPE, 1982a; ZAK, 1976) and field observations on fungal connections between fruiting bodies and roots. (ACSAI and LARGENT, 1983; ZAK, 1974). Arbutoid hosts appear to be less restrictive towards their fungal symbionts than ectomycorrhizal hosts. MOLINA and TRAPPE (1982a) found that 25/28 ectomycorrhizal fungi tested formed arbutoid mycorrhizas on *Arbutus menziesii* and *Arctostaphylos uva-ursi*, including many genus-specific fungi. However caution must be exercised in interpreting these results as high levels of carbohydrate were used in the synthesis medium. No indications of incompatibility were found in these arbutoid associations with conifer-specific fungi, as have been observed with other broad-leaved ectomycorrhizal hosts. *Leccinum manzanita*, a sporocarp-specific fungus of *Arbutus* and *Arctostaphylos* spp., has been found to form ectomycorrhizas with several conifers in aseptic culture (MOLINA and TRAPPE, 1982a). The majority of arbutoid hosts are recorded as forming typically arbutoid mycorrhizas but both ecto- (LARGENT *et al*., 1980) and ericoid (MEJSTRIK and HADAC, 1975) mycorrhizas have been described on some *Arbutus* and *Arctostaphylos* species. The fact that similar fungi can form ectomycorrhizas with one host and arbutoid mycorrhizas with another suggests that recognition may be host mediated.

SPECIFICITY IN ERICOID MYCORRHIZAS

Ericoid mycorrhizas are formed by a number of genera in the Ericaceae typified by *Calluna* , *Rhododendron*, *Erica* and *Vaccinium* species. Various Fungi Imperfecti eg. *Oidiodendron* and unidentified sterile mycelia have been isolated from ericaceous hosts by several workers and reinoculated to form ericoid mycorrhizas but it was not until an endophyte, isolated from *Calluna* (and since from other ericaceaous plants), was induced to fruit in culture, that an Ascomycete, now called *Pezizella ericae* (READ, 1974) was identified. The presence of simple septal pores and Woronin bodies indicate that most endophytes studied so far are Ascomycetes (BONFANTE-FASOLO and GIANINAZZI-PEARSON, 1979; DUDDRIDGE and READ, 1982a), although the occasional presence of dolipore septa in intracellular hyphae have implicated Basidiomycetes (BONFANTE-FASOLO, 1980; PETERSON *et al*., 1980). *Clavaria* spp. have been reported as ericoid endophytes but there is only indirect evidence for this (SEVIOUR *et al*., 1973; ENGLANDER and HULL, 1980) and isolation of the endophyte and resynthesis of the association is required to confirm it. None of the isolates appear to have any close host specificity as one isolate from one ericaceous species can form mycorrhizas with any other species. However there is no evidence that ericoid endophytes can form either ericoid or any other type of mycorrhiza on non-ericaceous hosts (BONFANTE-FASOLO *et al*., 1984; DUDDRIDGE unpub.). In this respect they have a much more limited host range than VAM fungi.

SPECIFICITY IN VESICULAR-ARBUSCULAR MYCORRHIZAS

Vesicular-arbuscular mycorrhizas (VAM) occur on the roots of most plants including the majority of angiosperms, some gymnosperms, pteridophytes and bryophytes. They have even been reported to form associations with plants which have been traditionally considered to be non-mycorrhizal. eg. certain plant families such as the Cruciferae and Chenopodiaceae, aquatic plants and members of the Coniferales. In contrast to their universality among higher plants, VAM endophytes are restricted to the family Endogonaceae in the Zygomycetes, the main genera being *Glomus*, *Acaulospora*, *Gigaspora*, and *Sclerocystis*. There is fossil evidence that fungi similar to VAM endophytes were associated with early land plants (*Asteroxylon* and *Rhynia*) 370 million years ago, but there appears to have been little co-evolution of the symbiosis towards a closer host specificity. Viable spores of these fungi are commonly found in soils where hosts have been grown or are growing and they readily infect host roots when present although they cannot be cultured *in vitro*. There appears to be no specificity at the host genus level as found with ectomycorrhizal symbionts. VAM fungi isolated from one host can, on the whole form mycorrhizas with a wide range of other hosts. For example MOLINA *et al*., (1978) tested 11 VAM fungi (including *Glomus*, *Acaulospora*, *Gigaspora* and *Sclerocystis* spp.) on 6 *Festuca* spp. and found no evidence of specificity of any endophyte for any particular *Festuca* species.

SPECIFICITY IN ORCHIDACEOUS MYCORRHIZAS

Fungi from the form-genus *Rhizoctonia*, which have perfect stages in the Tremellaceae and Tulasnellaceae are the most important symbionts of green orchids. Basidiomycetes have also been implicated in associations with some achlorophyllous orchids eg. *Armillaria mellea* agg. which has been shown to be an endophyte of *Gastrodia elata* (KUSANO, 1911) and *Galeola septrionalis* (SAGARA and TAKAJANA, 1978). In general there appears to be little specificity between orchids and their fungal symbionts. Hosts such as *Lyperanthus nigricans* (WARCUP, 1981) and *Dactylorchis purpurella* (HADLEY, 1970) form associations with a wide range of endophytes. As with ectomycorrhizal fungi, host specificity when it does occur, is mostly at the genus level e.g. *Caladenia* and *Sebacina vermifera*. This lack of specificity on the part of orchid endophytes is emphasised by the fact that several have proved to be parasitic on other hosts eg. *R. solani* and *A. mellea* agg., sometimes simultaneously with mycorrhizal formation (KUSANO, 1911). A Rhizoctonia species which is an orchid endophyte of *Rhizanthella gardneri* has been found to form ectomycorrhizas on *Melaleuca uncinata* and *Eucalyptus* (WARCUP, 1985b). The situation is further complicated by the fact that most data for specificity ha~~s~~ve been derived from germinating protocorms and not from adult roots where the situation may be different. Orchid endophytes isolated from adult plants are often unable to form a symbiotic association with the protocorm (HARVAIS and HADLEY, 1967). The fact that some orchid seeds have an obligate requirement for infection by a symbiont, yet do not carry it on their seed, suggests either that there is little or no host specificity (as with VAM fungi) or that the endophyte is widespread in the soil (WARCUP, 1981).

NON-HOSTS OF MYCORRHIZAL FUNGI

Non-hosts of mycorrhizal fungi include those plants that rarely form mycorrhizal associations and those that are hosts for one type of mycorrhizal fungus but non-hosts for any other type. VAM infection can be observed in some members of these typically non-mycorrhizal families both in the field, where the plants occur in an intensively competitive community of VAM and the inoculum density is therefore high (eg. READ *et al*., 1976; MILLER *et al*., 1983), and *in vitro* in the presence of a mycorrhizal companion plant (HIRREL *et al*., 1978; OCAMPO *et al*., 1980). Although the spores of VAM fungi will germinate (DANIELS and TRAPPE, 1980) and the hyphae grow (TOMMERUP, 1984) on the surface of non-host roots, there are fewer penetration points (OCAMPO *et al*., 1980) and arbuscules, although occasionally observed in field and pot-grown material (TOMMERUP, 1984), are rare. The presence of arbuscules is often used as an indicator of a functional VAM association (HIRREL *et al*., 1978), but they may only be present at a certain stage in the association and so their absence should not be used to question the mutualistic nature of the association. Ectomycorrhizal (FILER and TOOLE, 1966; THEODOROU and BOWEN, 1971; DUDDRIDGE, unpub.) and ericoid (BONFANTE-FASOLO *et al*., 1984; DUDDRIDGE, unpub.) fungi have also been observed growing on the surface of non-host roots. Penetration is rare and when it occurs it usually results in the death of host cells. In aseptic syntheses between mycorrhizal fungi and non-hosts the presence of glucose in the medium greatly increases the growth of the fungus in the rhizosphere (DUDDRIDGE, unpub.). In the presence of glucose ($10 gl^{-1}$)

S. grevillei, , an ectomycorrhizal symbiont with a narrow host range, is found to grow readily in the rhizosphere of non-hosts such as *Trifolium repens*, *Allium porrum* and *Rhododendron ponticum*. (Plate 1d) compared to syntheses where glucose is omitted (Plate 2c). The lack of specific stimulation by host plant roots and the ability of mycorrhizal fungi to grow in the rhizosphere of non-hosts may well be an adaptation on the part of the fungus to a very wide host range. Current research suggests that recognition or resistance of a non-host to a mycorrhizal fungus occurs after adhesion but before the formation of inter- or intracellular structures.

TISSUE SPECIFICITY

Mycorrhizal fungi, as their name implies, specifically infect roots. Inspite of the wide differences in the root tissue of such diverse ectomycorrhizal hosts as conifers, hardwoods, shrubs and herbs, the morphology of the mycorrhizas formed by the same fungus are similar on each eg. *Cenococcum graniforme* (TRAPPE, 1964). A sheath and Hartig net are even formed on actinorhizal nodules of *Alnus* by some ectomycorrhizal fungi (GODBOUT and FORTIN, 1983). The modification of the root tissue by *Frankia* does not appear to effect recognition. In most mycorrhizal associations penetration of the fungal symbiont occurs between or into cells that have just, or are just reaching maturity, in which primary cell wall building is still occurring. This is most obvious in the developing Hartig net of ectomycorrhizas, but in both VA (GIANINAZZI-PEARSON *et al*., 1980) and ericoid (PETERSON *et al*., 1980) mycorrhizas the first penetration points occur in the region just behind the apical meristem. SMITH and WALKER (1981) found that the root tip was ten times more likely to become infected than the rest of the root. It has been suggested in ectomycorrhizas (HARLEY and SMITH, 1983) that these cells in which wall formation is still active are susceptible to mycorrhizal infection and that the fungus in some way interferes with the host wall-building enzymes, allowing it to obtain wall precursors and resulting in the formation of the so-called involving layer (SCANNERINI, 1968; DUDDRIDGE and READ, 1984a and b). NYLUND (1981) suggested that cells in the 'mycorrhizal infection zone' are much higher in acid polysaccharides making them more flexible and therefore more susceptible to infection.

There are various tissue types in which mycorrhizal infection is believed not to occur and these include: (1) Actively photosynthetic tissue (2) Meristematic tissue (3) Suberised and secondary thickened tissue (4) Vascular tissue. There is circumstantial evidence that VAM fungi may form arbuscules in photosynthetic tissue of bryophytes (GOURRET and STRULLU, 1979; POCOCK and DUCKETT, 1984), although the invasion of green tissue usually results in chloroplast breakdown (HADLEY and WILLIAMSON, 1971).

RECOGNITION IN MYCORRHIZAL ASSOCIATIONS

An area of mycorrhizal research which has been virtually ignored is how mycorrhizal symbionts recognise each other. In plant-pathogen interactions recognition usually occurs for resistance i.e. a host has receptors on the surface capable of recognising specific markers on the fungal surface which alert the defence system and results in resistance. Those hosts which lack the receptors for the pathogen markers are susceptible because they do not activate the defence system (ALBERSHIEM and ANDERSON-PROUTY, 1975). In interaction between *Rhizobium* spp. and legumes however, recognition appears to be for compatibility (BAUER, 1981). So how do mycorrhizal symbionts recognise each other? There are two possible explanations (a) Fungi are recognised as mycorrhizal symbionts, thus ensuring compatibility or (b) Specific recognition of mycorrhizal fungi does not occur because they do not trigger the host defence system unless they interact with an incompatible host or a non-host. One fact is certain, whether recognition occurs for compatibility or incompatibility, it is not a 'once and for all' process and it is the early events immediately preceding and during contact that determine whether the symbiosis develops or not. Recognition must therefore involve a series of steps and the sequence may be halted at any one of these.

In most cases the stimulation of spore germination and the initial attraction of mycorrhizal fungi to plant roots appears to be non-specific, as both can occur in the rhizosphere of non-hosts. Indeed the attraction of fungi to plant roots because of the increased availability of nutrients provided by root exudates and sloughed off cells, is not restricted to mycorrhizal fungi but includes many saprophytes and parasites, although only the former are able to penetrate and form close symbiotic associations with roots. At some stage between the growth of mycorrhizal fungi in the rhizosphere and the development of intra- or inter-cellular infection structures, recognition for compatibility or incompatibility occurs. Hyphal growth and the formation of various infection structures of both root (GARRETT, 1956) and shoot (STAPLES and MACKO, 1980) infecting fungi are often concentrated at cell junctions, areas of high exudation. This phenomenon although marked in the early development of some arbutoid mycorrhizas of *Pyrola rotundifolia* (Plate 2g) is not so obvious in the formation of ectomycorrhizas. As observed in plant-pathogen interactions (STAPLES and MACKO, 1980) contact between the symbionts is tight in compatible associations and loose where the fungus is growing on an incompatible host or a non-host (THEODOROU and BOWEN 1971; DUDDRIDGE unpub.). A mucilaginous substance, which sometimes has a fibrillar ultrastructure, has been observed attaching both pathogenic (MURRAY and MAXWELL, 1975) and mycorrhizal (DUDDRIDGE, 1985) fungi to plant surfaces (Plates 2b and d). In ericoid mycorrhizas it was originally thought to be involved in recognition as it was not produced in the absence of the host (BONFANTE-FASOLO and GIANINAZZI-PEARSON, 1982) but has recently been found binding hyphae to the surface of non-hosts of both ericoid (BONFANTE-FASOLO *et al.*, 1984) and ectomycorrhizal (DUDDRIDGE, 1985) fungi (Plate 2a) and is probably a non-specific attachment mechanism.

Modification of the cell walls of both plant and fungal symbionts occurs during the formation of both ecto- and endo- mycorrhizas and is obviously important in the successful establishment of the symbiosis. These morphological changes are obvious manifestations of interactions between complementary surface components. Carbohydrate-binding proteins called lectins have been frequently implicated in recognition (CALLOW, 1977; SCHMIDT, 1979), although there are only a few hard facts of their involvement in plant-microbe symbioses (BOHLOOL and SCHMIDT, 1974). Modifications of the cell walls and membranes in VAM are discussed in detail by Bonfante-Fasolo, in this volume. In ectomycorrhizas, contact between the symbionts results in alterations both in the cell walls of the symbionts and the mode of growth of the fungus. During the formation of the Hartig net the adjacent fungal and host walls lose their integrity and become indistinguishable from each other forming an electron-opaque matrix known as the involving layer (Plate 2f). At some stage during early mycorrhizal development the hyphal morphology of the fungal symbiont is altered and tissue formation is induced in both the inner sheath and Hartig net, giving the later its characteristic labrynthine appearance on the surface of cortical cells (Plate 2e). This change in morphology which occurs in taxonomically diverse fungi, is presumably host-induced and may be the first evidence of the host's response to fungal infection (NYLUND and UNESTAM, 1982). Although mycorrhizal fungi are frequently able to grow in the rhizosphere of both incompatible hosts and non-hosts, a compact sheath and Hartig net are rarely formed. Thus it seems that a functional mycorrhiza will not form on an incompatible or a non-host because the fungus is recognised as incompatible, preventing further development of the host-fungus interface at either the inter or intra -cellular level. The hosts response to an incompatible mycorrhizal symbiont is similar to that produced by fungal pathogens on a non-susceptible or immune host (MOLINA and TRAPPE, 1982b; DUDDRIDGE in prep.). Unlike the highly specific interactions that occur between biotrophic parasites and their hosts on which much of the work of recognition has been done, mycorrhizal associations create a number of problems. They are on the whole non-specific interactions, one host forming mycorrhizas with a large number of fungi and one fungus interacting with a wide spectrum of hosts. This makes the search for particular molecules which are involved in recognition very difficult. The situation is complicated even further by the fact that many ectomycorrhizal fungi which produce intercellular infections on ectomycorrhizal hosts can produce both inter- and intra- cellular infections on arbutoid hosts. What mechanism allows the fungus to penetrate into these particular host cells and not into others? How do fungi which are normally plant parasites (eg. *Rhizoctonia solani* and *Armillaria mellea*) become mycorrhizal endophytes of orchids? What allows a fungus to form ectomycorrhizas on one host and orchidaceous, arbutoid or monotropoid mycorrhizas on another? How can one host form ectomycorrhizas under one set of conditions and endomycorrhizas under others? These complications may be one of the reasons why research workers have steered well clear of recognition in mycorrhizal associations. We need to systematically characterise the chemical, ultrastructural and enzymic changes that occur in the walls and membranes of the symbionts during contact and to study the interaction of mycorrhizal fungi on both incompatible hosts and non-hosts to observe at what stage resistance occurs and what form it takes. We must not be too proud to coerce or cajole workers with expertise from relevant fields such as plant cell wall biochemistry, plant pathology and immunology to co-operate in joint projects which will eventually enable us to answer some of the questions concerning recognition in mycorrhizal symbioses.

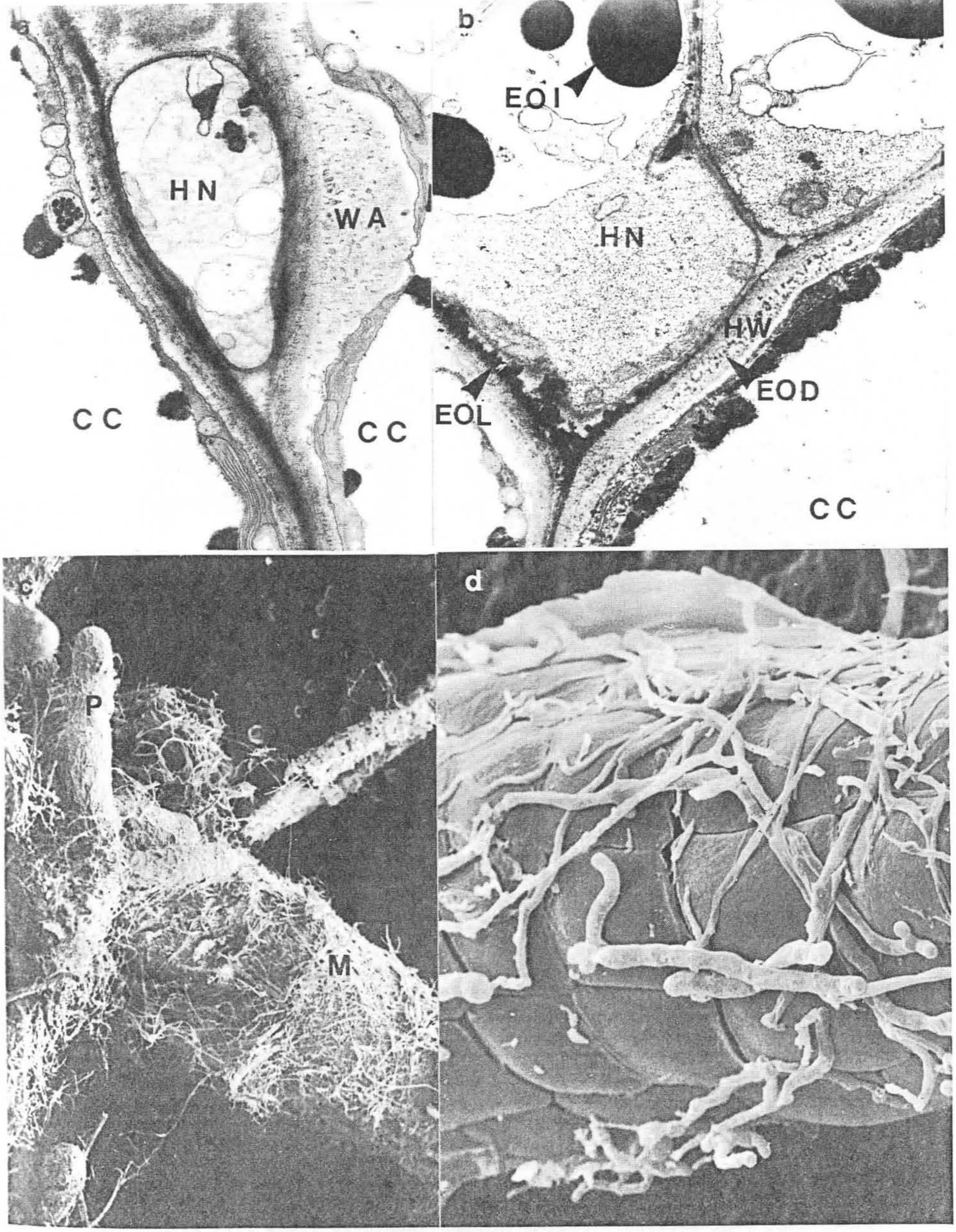
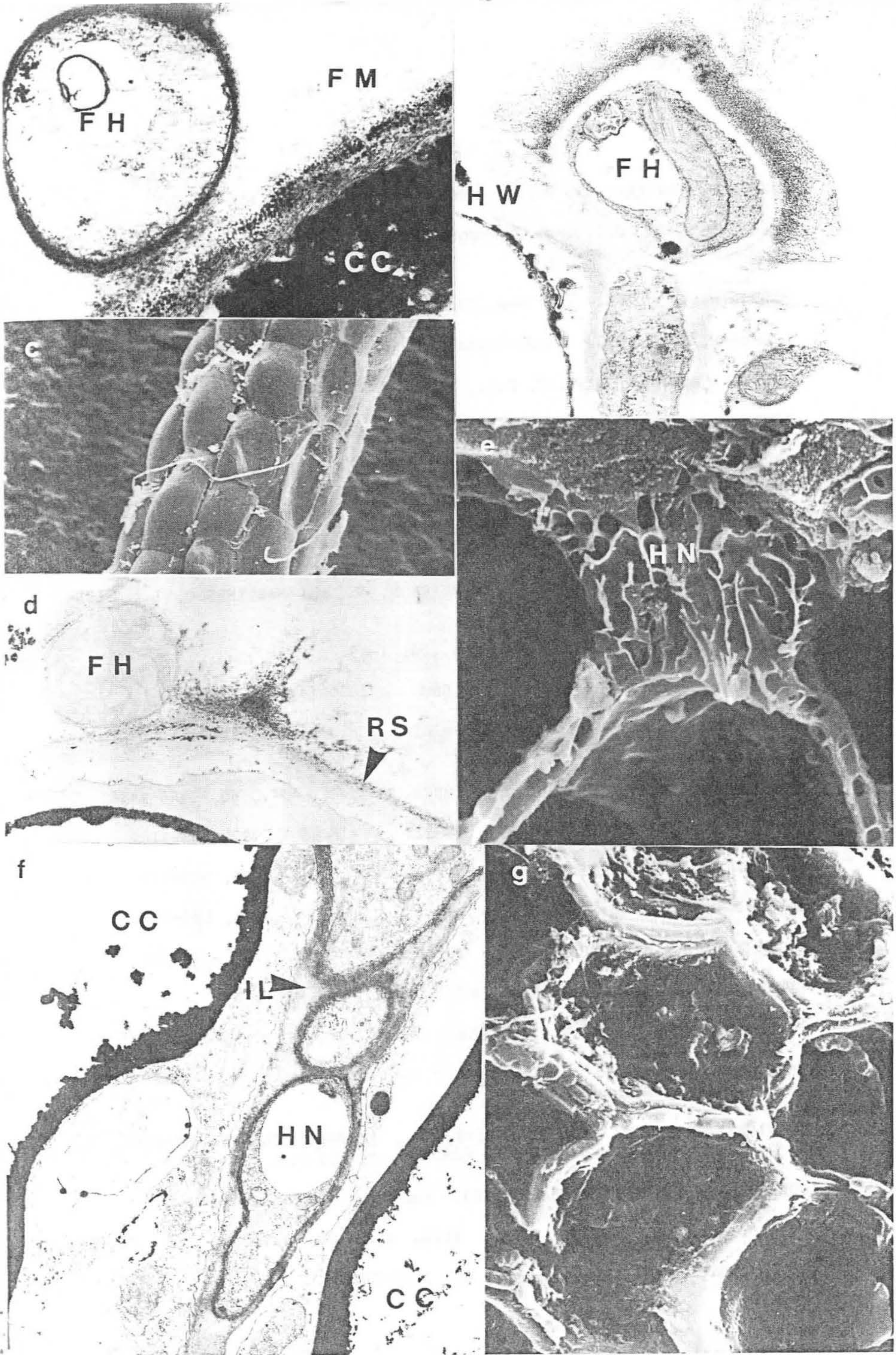


Plate 1. (a) and (b) Transmission electron micrographs (TEM) of mycorrhizas synthesised between *P. menziesii* and *S. grevillei*. (a) Wall apposition (WA) on cortical cell (CC) adjacent to the Hartig net (HN). X 15,000. (b) Host wall (HW) thickening and electron opaque deposits (EOD) in wall adjacent to the Hartig net; electron opaque layer (EOL) between the host and fungal walls and electron opaque inclusions (EOI) in fungus. X 12,500. (c) Mycorrhizas of *Pinus* (P) and *Monotropa* (M) bound together by common fungal symbiont. X 75. (d) Scanning electron micrograph (SEM) showing hyphae of *S. grevillei* growing on the surface of *Rhododendron ponticum* (10gl^{-1} glucose in synthesis medium). X 10,000.

Plate 2. (a) TEM of hypha (FH) of *S. grevillei* attached to root surface of *R. ponticum* by fibrillar material (FM) (10gl^{-1} glucose in synthesis medium). X 25,000. (b) TEM of *P. ericae* hypha attached by fibrillar material (FM) to the cell wall (HW) of *Calluna vulgaris*. X 20,000 (Photo courtesy of V. Gianinazzi-Pearson). (c) SEM of *Larix decidua* hyphae on the root surface of *R. ponticum* (no glucose in synthesis medium). X 1,375. (d) TEM of *S. grevillei* hypha (FH) attached to surface (RS) *Larix decidua* root. Stained with Thiéry's procedure (also 1(a)) for the localisation of periodate-sensitive carbohydrates. X 12,500 (e) SEM of a transverse fracture of an ectomycorrhiza showing the characteristic labrythine development of the fungal tissue in the Hartig net (HN) on the surface of a cortical cell. X 1,875. (f) TEM of an ectomycorrhiza of *Pinus contorta*, showing a well-developed involving layer (IL) between the cortical cells (CC) and the Hartig net (HN). X 9,150. (g) SEM of an arbutoid mycorrhiza of *Pyrola rotundifolia* showing the growth of the symbiont along the cell junctions of the host. X 1,760.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